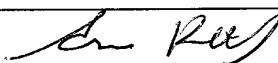


U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 10-95)		ATTORNEY'S DOCKET NUMBER <b>10391/003001</b>
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR <b>09/171740</b>
INTERNATIONAL APPLICATION NO. <b>PCT/IE97/00030</b>	INTERNATIONAL FILING DATE <b>APRIL 22, 1997</b>	PRIORITY DATE CLAIMED <b>APRIL 23, 1996</b>
TITLE OF INVENTION <b>SUSTAINED RELEASE IONIC CONJUGATE</b>		
APPLICANT(S) FOR DO/EO/US <b>FRANCIS IGNATIOUS, THOMAS CIARAN LOUGHMAN, SHALABY W. SHALABY AND FRANCK JEAN-CLAUDE TOURAUD</b>		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> <li><input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li><input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li><input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2))             <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li><input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li><input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).</li> <li><input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li><input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li><input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).</li> <li><input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).</li> <li><input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> </ol>		
Items 13 to 18 below concern document(s) or information included:		
<ol style="list-style-type: none"> <li><input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li><input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li><input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment. A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li><input type="checkbox"/> A substitute specification.</li> <li><input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li><input checked="" type="checkbox"/> Certificate of Mailing by Express Mail</li> <li><input type="checkbox"/> Other items or information:</li> </ol>		
<p>"Express Mail" label number : EL010096445US  <b>Date of Deposit : OCTOBER 23, 1998</b></p> <p>I hereby certify that under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office To Addressee" with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.</p> 		

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/IE97/00030	10391/003001

20. The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

<input checked="" type="checkbox"/> Search Report has been prepared by the EPO or JPO .....	\$930.00
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) .....	\$720.00
<input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....	\$790.00
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....	\$1,070.00
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....	\$98.00

**CALCULATIONS PTO USE ONLY**

**ENTER APPROPRIATE BASIC FEE AMOUNT =**

**\$930.00**

Surcharge of **\$130.00** for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)).

20     30

**\$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	65 - 20 =	45	x \$22.00	<b>\$990.00</b>
Independent claims	4 - 3 =	1	x \$82.00	<b>\$82.00</b>
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	<b>\$0.00</b>

**TOTAL OF ABOVE CALCULATIONS =**

**\$2,002.00**

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable).

**\$0.00**

**SUBTOTAL =**

**\$2,002.00**

Processing fee of **\$130.00** for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)).

20     30

**\$0.00**

**TOTAL NATIONAL FEE =**

**\$2,002.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).

**\$0.00**

**TOTAL FEES ENCLOSED =**

**\$2,002.00**

Amount to be: **\$**

refunded

charged **\$**

A check in the amount of **\$2,002.00** to cover the above fees is enclosed.

Please charge my Deposit Account No. in the amount of to cover the above fees.  
A duplicate copy of this sheet is enclosed.

The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **06-1050** A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

Y. Rocky Tsao Ph.D.  
Fish & Richardson PC  
225 Franklin Street  
Boston, Massachusetts 02110

  
SIGNATURE

Y. Rocky Tsao Ph.D.

NAME

34,053

REGISTRATION NUMBER

OCTOBER 23, 1998

DATE

09/171740

405 Rec'd PCT/PTO 23 OCT 1998

PATENT  
ATTORNEY DOCKET NO. 10391/003001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Francis Ignatious et al. Art Unit:  
Serial No.: Examiner:  
Filed : HEREWITH  
Title : SUSTAINED RELEASE IONIC CONJUGATE

Int'l Appln. No. : PCT/IE97/00030  
Int'l Filing Date: April 22, 1997

**Box PCT**  
Assistant Commissioner for Patents  
Washington, DC 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the Specification:

Page 1, before the first line, please insert the following paragraph: --This is a continuation of International Patent Application No. PCT/IE97/00030, with an international filing date of April 22, 1997, now pending.--

In the Claims:

Claim 4, line 1, delete "any one of claims 1-3" and insert --claim 1--.

Claim 5, line 1, delete "any preceding claim" and insert --claim 1--.

Claim 6, line 1, delete "any preceding claim" and insert --claim 1--.

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J. Rus  
Anne Ry

Claim 7, line 1, delete "any preceding claim" and  
insert --claim 1--.

Claim 9, line 1, delete "any one of claim 4-8" and  
insert --claim 4--.

Claim 10, line 1, delete "any preceding claim" and  
insert --claim 1--.

Claim 12, line 1, delete "any preceding claim" and  
insert --claim 1--.

Claim 13, line 1, delete "any preceding claim" and  
insert --claim 1--.

Claim 15, line 1, delete "or 14".

Claim 16, line 1, delete "any one of claim 1-12" and  
insert --claim 1--.

Claim 19, line 1, delete "any preceding claim" and  
insert --claim 1--.

Claim 24, line 1, delete "or 23".

Claim 25, line 1, delete "any one of claim 22-25" and  
insert --claim 22--.

Claim 26, line 1, delete "any one of claim 22-25" and  
insert --claim 22--.

Claim 27, line 1, delete "any one of claim 22-26" and  
insert --claim 22--.

Claim 31, line 1, delete "or 30".

Claim 33, line 1, delete "any one of claim 29-32" and  
insert --claim 29--.

Claim 34, line 1, delete "any one of claim 29-33" and  
insert --claim 29--.

Claim 35, line 1, delete "any one of claim 29-34" and  
insert --claim 29--.

Claim 37, line 1, delete "any one of claim 29-36" and  
insert --claim 29--.

Cancel claims 38 and 39.

Add new claims 40 to 67.

-- 40. A method according to claim 8, wherein said  
polyester comprises lactic acid, glycolic acid and tartaric acid.

41. Biodegradable polymer comprising lactic acid,  $\epsilon$ -  
caproic acid, glycolic acid, trimethylene carbonate, p-dioxanone  
or a copolymer thereof and tartaric acid.

42. Microparticles comprising a biodegradable polymer  
according to claim 41.

43. Microparticles of a sustained release ionic  
conjugate comprising the biodegradable polymer according to claim  
41 and a drug containing one or more free amino groups, wherein  
the polymer and drug are ionically bonded.

44. Microparticles according to claim 43 wherein said  
drug is selected from the group consisting of growth hormone  
releasing peptide luteinizing hormone-releasing hormone,  
adrenomedullin, growth hormone, somatostatin, bombesin, gastrin  
releasing peptide, calcitonin, bradykinin, galanin, melanocyte  
stimulating hormone, growth hormone releasing factor, amylin,

tachykinins, secretin, parathyroid hormone, enkephalin, endothelin, calcitonin gene releasing peptide, neuromedins, parathyroid hormone related protein, glucagon, neuropeptides, adrenocorticotrophic hormone, peptide YY, glucagon releasing peptide, vasoactive intestinal peptide, pituitary adenylated cyclase activating peptide, motilin, substance P, neuropeptide Y and TSH or an analogue or a fragment thereof.

45. Microparticles according to claim 44 wherein said drug is somatostatin or LHRH or an analogue or a fragment thereof.

46. Microparticles according to claim 45 wherein said somatostatin analogue is D- $\beta$ -Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-NH<sub>2</sub>.

47. The biodegradable polymer according to claim 41 comprising lactic acid glycolic acid and tartaric acid.

48. Microparticles comprising a biodegradable polymer according to claim 47.

49. Microparticles of a sustained release ionic conjugate comprising the biodegradable polymer according to claim 47 and a drug containing one or more free amino groups, wherein the polymer and drug are ionically bonded.

50. Microparticles according to claim 49 wherein said drug is selected from the group consisting of growth hormone releasing peptide, luteinizing hormone-releasing hormone, adrenomedullin, growth hormone, somatostatin, bombesin, gastrin releasing peptide, calcitonin, bradykinin, galanin, melanocyte stimulating hormone, growth hormone releasing factor, amylin, tachykinins, secretin, parathyroid hormone, enkephalin, endothelin, calcitonin gene releasing peptide, neuromedins, parathyroid hormone related protein, glucagon, neuropeptid Y, adrenocorticotrophic hormone, peptide YY, glucagon releasing peptide, vasoactive intestinal peptide, pituitary adenylated cyclase activating peptide, motilin, substance P, neuropeptide Y and TSH or an analogue or a fragment thereof.

51. Microparticles according to claim 50 wherein said drug is somatostatin or LHRH or an analogue or a fragment thereof.

52. Microparticles according to claim 51 wherein said somatostatin analogue is D- $\beta$ -Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-NH<sub>2</sub>.

53. The biodegradable polymer according to claim 47 wherein the ratio of lactic acid to glycolic acid to taratic acid is about 66 to about 33 to about 1, respectively.

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55. Microparticles of a sustained release ionic conjugate comprising the biodegradable polymer according to claim 53 and a drug containing one or more free amino groups, wherein the polymer and drug are ionically bonded.

56. Microparticles according to claim 55 wherein said drug is selected from the group consisting of growth hormone releasing peptide, luteinizing hormone-releasing hormone, adrenomedullin, growth hormone, somatostatin, bombesin, gastrin releasing peptide, calcitonin, bradykinin, galanin, melanocyte stimulating hormone, growth hormone releasing factor, amylin, tachykinins, secretin, parathyroid hormone, enkephalin, endothelin, calcitonin gene releasing peptide, neuromedins, parathyroid hormone related protein, glucagon, neuropeptid Y, adrenocorticotrophic hormone, peptide YY, glucagon releasing peptide, vasoactive intestinal peptide, pituitary adenylated cyclase activating peptide, motilin, substance P, neuropeptide Y and TSH or an analogue or a fragment thereof.

57. Microparticles according to claim 56 wherein said drug is somatostatin or LHRH or an analogue or a fragment thereof.

58. Microparticles according to claim 57 wherein said somatostatin analogue is D- $\beta$ -Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-NH<sub>2</sub>.

59. The biodegradable polymer according to claim 47  
wherein the ratio of lactic acid to glycolic acid to taratic acid  
is about 66 to about 32 to about 2, respectively.

60. Microparticles comprising a biodegradable polymer  
according to claim 59.

61. Microparticles of a sustained release ionic  
conjugate comprising the biodegradable polymer according to claim  
60 and a drug containing one or more free amino groups, wherein  
the polymer and drug are ionically bonded.

62. Microparticles according to claim 61 wherein said  
drug is selected from the group consisting of growth hormone  
releasing peptide, luteinizing hormone-releasing hormone,  
adrenomedullin, growth hormone, somatostatin, bombesin, gastrin  
releasing peptide, calcitonin, bradykinin, galanin, melanocyte  
stimulating hormone, growth hormone releasing factor, amylin,  
tachykinins, secretin, parathyroid hormone, enkephalin,  
endothelin, calcitonin gene releasing peptide, neuromedins,  
parathyroid hormone related protein, glucagon, neurotensin,  
adrenocorticotropic hormone, peptide YY, glucagon releasing  
peptide, vasoactive intestinal peptide, pituitary adenylylated  
cyclase activating peptide, motilin, substance P, neuropeptide Y  
and TSH or an analogue or a fragment thereof.

63. Microparticles according to claim 62 wherein said drug is somatostatin or LHRH or an analogue or a fragment thereof.

64. Microparticles according to claim 63 wherein said somatostatin analogue is D- $\beta$ -Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-NH<sub>2</sub>.

65. A method according to claim 21 wherein said polyester comprises lactic acid, glycolic acid and tartaric acid.

66. A method according to claim 28 wherein said polyester comprises lactic acid, glycolic acid and tartaric acid.

67. A method according to claim 37 wherein said polyester comprises lactic acid, glycolic acid and tartaric acid.--

REMARKS

The specification is amended to recite claimed benefit under 35 USC 120 from International Patent Application PCT/IE97/00030.

Applicant has amended the dependency of the claims to preclude a rejection under 35 U.S.C. § 112, fifth paragraph, which provides in part that "[a] multiple dependent claim shall not serve as a basis for any other multiple dependent claim." No new matter has been added by the above amendments.

For the Examiner's convenience, additional pages for the specification, i.e., pages 23 to 27 containing new claims 40 to 67, are also filed herewith.

Please apply any charges not covered, or any credits, to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 10-23-98

Y. Rocky Tsoo  
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336572.B11

09/171740  
73 Rec'd PCT/PIC 23 OCT 1998

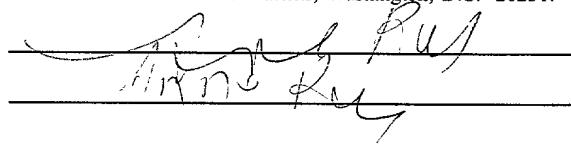
**APPLICATION  
FOR  
UNITED STATES LETTERS PATENT**

**TITLE:** SUSTAINED RELEASE IONIC CONJUGATE  
**APPLICANT:** FRANCIS IGNATIOUS, THOMAS CIARAN LOUGHMAN,  
SHALABY W. SHALABY AND FRANCK JEAN-CLAUDE  
TOURAUD

"EXPRESS MAIL" Mailing Label Number EL010096445US

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Assistant Commissioner for Patents, Washington, D.C. 20231.



Description /Sustained release ionic conjugateTechnical Field

This invention relates to sustained release drug delivery systems  
5 and, in particular, to a method of making microparticles of a sustained  
release ionic conjugate.

Background Art

Biodegradable polymeric drug delivery formulations have been  
developed and utilized for the controlled *in vivo* release of drugs. See,  
10 e.g., U.S. Patent Nos. 3,773,919 and 4,767,628. Such biodegradable  
polymeric formulations are designed to allow an entrapped drug to  
slowly diffuse through a polymer matrix or coating when the  
biodegradable polymer is depolymerized.

15 International Publication No. WO 94/15587 describes sustained  
release ionic molecular conjugates of polyesters and drugs. Since  
polyester degradation is a key step in the release process, the surface  
area of the conjugate particles can control the release profile of the  
drug from the conjugate. Thus, the conjugate particles should be of  
similar size and shape to insure both the minimum and reproducible  
20 surface area, e.g., microspheres.

Disclosure of Invention

In one aspect, this invention features a method of making  
microparticles of a sustained release ionic conjugate containing a free  
carboxyl group-containing biodegradable polymer (a polyester made of  
25 monomers such as lactic acid, *e*-caprolic acid, glycolic acid,  
trimethylene carbonate, or *p*-dioxanone; or a copolymer thereof; the  
monomers can be optically isomers or racemates) and a free amino  
group-containing drug (e.g., a peptide drug such as somatostatin or

LHRH) which are ionically bonded to each other. The method includes the steps of (1) obtaining a first solution in which the conjugate is dissolved; (2) mixing the first solution (added as small droplets, e.g., through an atomizing nozzle such as sonic nozzle, pneumatic nozzle, 5 rotary atomizer, or pressure nozzle) with a first liquid to form a first dispersion, wherein the first liquid is miscible with the first solution, and the conjugate is not soluble in the first liquid and precipitates out of the first dispersion; and (3) isolating the conjugate from the first dispersion.

10 In one embodiment, the drug is soluble in the first liquid, which can be an alcohol (e.g., ethanol or isopropyl alcohol), hexane, or water; or a mixture thereof. When ethanol is used as the first liquid, it can be maintained between about 0°C and -30°C when it is being used. When isopropyl alcohol is used, it can be maintained between about 0°C and 15 -70°C, e.g., cooled by the addition of dry ice.

The first solution, which may contain acetone, dichloromethane, acetonitrile, ethyl acetate, tetrahydrofuran, or glyme, or mixtures thereof can be obtained by (1) dissolving the biodegradable polymer in a second liquid (e.g., acetone, tetrahydrofuran, glycine, ethyl acetate, 20 methyl acetate, acetonitrile, ethyl formate, or glyme; or a mixture thereof) to form a second solution; (2) dissolving the drug in a third liquid (e.g., water or acetone; or a mixture thereof) to form a third solution, wherein the third liquid is miscible with the first liquid and the second liquid; and (3) mixing the second solution and the third 25 solution to form the first solution, wherein the mixing causes the drug to ionically bond to the biodegradable polymer and form the conjugate in the first solution. The first solution may comprise up to 40% by weight of the conjugate (e.g., between 25 and 35 percent by weight of the conjugate). In one example, a base, e.g., NaOH or KOH, can be 30 added to the second solution prior to mixing the second solution and the third solution. Neutralization of the carboxyl groups of the biodegradable polymer with the base facilitates the formation of the ionic conjugate.

Alternatively, the first solution is obtained by dissolving the biodegradable polymer and the drug in a second liquid (e.g., acetone or a mixture of acetone and water) to form the first solution, thereby forming the conjugate in the first solution. According to this method, 5 the biodegradable polymer can be first dissolved in the second liquid, a base is then added to the second solution, and the drug is subsequently dissolved in the second liquid. Also, if desired, the first solution can be partially or completely evaporated from the first dispersion prior to isolation of the conjugate. The processed conjugate can be conveniently 10 isolated by centrifuging or filtering the first dispersion, and the isolated conjugate can be mixed with an aqueous mannitol solution prior to vacuum drying (e.g., lyophilization). The isolated conjugate can be further shaped into a film or a rod. The isolated conjugate can also be spherified into microspheres of average diameter of 5 to 200  $\mu\text{m}$ , e.g., 15 as described herein. By "spherification" or "spherifying" is meant the processing of a microparticle into a shape close to a sphere.

In another aspect, this invention features a method of spherifying a sustained release ionic conjugate as described above. The method includes the steps of (1) mixing the conjugate with a first liquid (e.g., 20 an oil such as silicon oil, mineral oil, sesame oil, or a vegetable oil) to form a first dispersion, wherein the conjugate has the shape of a microparticle and is not soluble in the first liquid; (2) heating the first dispersion to a temperature greater than the Tg or Tm of the conjugate; (3) cooling the first dispersion below the Tg or Tm of the conjugate; 25 (4) mixing the first dispersion with a second liquid (e.g., hexane, heptane, isopropyl myristate, or an alcohol such as ethanol or isopropyl alcohol) to form a second dispersion, wherein the second liquid is miscible with the first liquid and the conjugate is not soluble in the second liquid; and (5) isolating the conjugate from the second dispersion. The conjugate may have the shape of a microcapsule with 30 an average diameter of between 5  $\mu\text{m}$  to 200  $\mu\text{m}$  prior to mixing with the first liquid, and the first dispersion thus formed is vigorously stirred while being heated to aid in the separation of the particles. Once the conjugate has been isolated, it can be rinsed with the second

liquid and then vacuum dried. Optionally, it can also be mixed with an aqueous mannitol solution prior to vacuum drying.

A third aspect of this invention features a method of spherifying the above-described sustained release ionic conjugate (e.g., a microcapsule having an average diameter of between 5  $\mu\text{m}$  to 200  $\mu\text{m}$ ). The method includes the steps of (1) mixing the conjugate in a first liquid (e.g., water) to form a first dispersion, wherein the conjugate is in the shape of a microparticle and the conjugate is not soluble in the first liquid; (2) stirring the first dispersion; (3) mixing the stirred dispersion with a second liquid (e.g., dichloromethane or chloroform) in such an amount so that it is absorbed by the conjugate but does not solubilize the conjugate, wherein the second liquid is miscible with the first liquid; (4) evaporating the second liquid from the first dispersion; and (5) isolating the precipitated conjugate from the first dispersion. If necessary, the method may further include the step of adding a surfactant (e.g., lecithin, Tween 20, polysorbate, or lauryl sulfate) to the first dispersion to aid in the stabilization of the first dispersion, and the isolated conjugate can be rinsed with the first liquid and vacuum dried. Again, the isolated conjugate can be mixed with an aqueous mannitol solution prior to vacuum drying.

In a further aspect of this invention, this invention features a method of spherifying the above-described sustained release ionic conjugate. The method includes the steps of (1) dissolving the conjugate in a first liquid (e.g., acetonitrile) to form a first solution; (2) stirring the first solution with a second liquid (e.g., an oil) to form a first dispersion, wherein the second liquid is immiscible with the first solution; (3) evaporating the first liquid from the first dispersion to precipitate the conjugate from the first dispersion; and (4) isolating the precipitate conjugate from the first dispersion. In the stirring step, the first solution can be added to the second liquid as small droplets.

The above method can further include the step of rinsing the isolated conjugate with a third liquid (e.g., hexane, heptane, or octane) which is miscible with the second liquid and not a solvent for the

isolated conjugate. If desired, the isolated conjugate can be mixed with an aqueous mannitol solution prior to vacuum drying.

The biodegradable polymer in the above-described conjugate may contain at least one free carboxyl group (e.g., two to ten free carboxyl groups per polymer chain). Examples of carboxylic acid containing biodegradable polymers include polyesters containing units of lactic acid, *e*-caprolic acid, *p*-dioxanone, *e*-caprionic acid, substituted and unsubstituted trimethylene carbonate, 1,5-dioxepan-2-one, 1,4-dioxepan-2-one, glycolic acid, alkylene oxylate, cycloalkylene, cycloalkylene oxylate, alkylene succinate, or 3-hydroxy butyrate in optically active forms or as racemates; or copolymers of any of the above. Additional free carboxylic acid groups can be incorporated into the biodegradable polyester by reaction, e.g., ring opening polymerization or polycondensation, with polycarboxylic acids such as malic acid, tartaric acid, pamoic acid, citric acid, succinic anhydride, and glutaric anhydride. Thus, the biodegradable polymer can be a water insoluble polyester including lactic acid units with or without glycolic acid units. Other biodegradable polymers such as polyorthoesters, polyorthocarbonates, and polyantals may also be used. The biodegradable polymer may have an average degree of polymerization, e.g., average number of monomers per polymer chain, between 10 and 300.

The drug has one or more (e.g., one to ten) free amine groups. In one embodiment, the drug is an acid-stable peptide. Examples of suitable acid-stable peptides include growth hormone releasing peptide (GHRP), luteinizing hormone-releasing hormone (LHRH), adrenomedullin, growth hormone, somatostatin, bombesin, gastrin releasing peptide (GRP), calcitonin, bradykinin, galanin, melanocyte stimulating hormone (MSH), growth hormone releasing factor (GRF), amylin, adrenomedullin, tachykinins, secretin, parathyroid hormone (PTH), enkephalin, endothelin, calcitonin gene releasing peptide (CGRP), neuromedins, parathyroid hormone related protein (PThrP), glucagon, neuropeptides Y and YY (PYY), glucagon releasing peptide (GLP), vasoactive intestinal

S E C R E T I O N G R A M P A T E

peptide (VIP), pituitary adenylated cyclase activating peptide (PACAP), motilin, substance P, neuropeptide Y (NPY), TSH, and analogs and fragments thereof. The drug may be soluble (e.g., greater than 0.1 mg/ml; preferably, greater than 1.0 mg/ml) in the first liquid.

5        Other features and advantages of the present invention will be apparent from the detailed description and from the claims.

It is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

10      Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

#### Best Modes for Carrying Out the Invention

##### Example 1

15      18.0 g of a 6,000 g/mol 66/32/2 poly-L-lactic-co-glycolic-co-D,L-malic acid copolymer (66 percent L-lactic acid, 32 percent glycolic, and 2 percent malic acid; acid number of 0.373 milliequivalents/g) was dissolved in 180 g of acetone (solution of 10% copolymer by weight). 14.4 ml of 0.5 N aqueous NaOH was added to form the sodium carboxylate of the polymer. 4.28 g of the acetate salt of the peptide Lanreotide™ (Kinerton, Dublin, Ireland; D-Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-NH<sub>2</sub>; acetate content = 9.60 percent by weight) was separately dissolved in a mixture of 10 g of acetone and 10 g of deionized water. The amount of peptide dissolved corresponded to the stoichiometric ratio of acid groups from the copolymer (e.g., one) and the free amino groups for the peptide (e.g., two). The peptide

solution was then added dropwise to the copolymer solution, and the resulting solution was stirred for two hours to allow for salt exchange and the resulting formation of the polymer/peptide ionic conjugate (PPIC).

5

### Example 2

In a temperature controlled jacketed reactor (Schott Glass AGB, Dublin, Ireland), a two liter bath of deionized water was precooled to 0°C and was vigorously stirred. The above PPIC solution of Example 1 was then slowly added to the reactor using a Masterflex pump (Bioblock Scientific, Illkivch, France) which produced a flow rate of 10-15 ml/min through a silicone tubing fitted with a 19 gauge needle at its tip. The PPIC solution was fed through the needle that was positioned above a water bath at 0°C. The PPIC precipitated in the bath as small, solid particles. The solid particles were then separated from the supernatant by centrifugation (30 minutes at 5000 rpm and 0-5°C), rinsed with fresh deionized water, resuspended water, recentrifuged, and then lyophilized. The isolated conjugate was filtered through a 100 µm sieve to remove any large particles which would not be capable of being injected through a 21 gauge needle. An analysis of the resulting particle sizes is described in Table I.

25

### Example 3

The PPIC solution of Example 1 was also precipitated as described above in Example 2, except that a bath of ethanol at a temperature of -20°C was used instead of a bath of water at 0°C. An analysis of the resulting particle sizes is described in Table I.

25

### Example 4

30

The PPIC solution of Example 1 was also dispersed at a controlled flow rate of 4 ml/min through an atomizing nozzle containing a hollow tip (Bioblock; 50 Watts, 20 kHz) over a bath of ethanol at -10°C in a temperature controlled jacket reactor. In this

nebulization process, the copolymer solution was released from the probe as a fine mist of small droplets. The small droplets fell into the ethanol bath, causing the deionized water and acetone to be extracted from the droplets. As a result, the copolymer droplets hardened as small, solid particles. The particles were then recovered by centrifugation and lyophilized. An analysis of the resulting particle sizes is listed in Table I. What is meant by Diameter -10 (i.e., D0.1), Diameter -50 (i.e., D0.5), or Diameter -90 (i.e., D0.9) is the smallest diameter which is greater than 10%, 50%, and 90% of the total particles, respectively. What is meant by specific area is the average specific area of the resulting particles.

TABLE I

	Example	Diameter-10 ( $\mu\text{m}$ )	Diameter-50 ( $\mu\text{m}$ )	Diameter-90 ( $\mu\text{m}$ )	Specific Area ( $\text{m}^2/\text{g}$ )
15	2	10	30	62	18.64
	3	9	37	89	6.42
	4	13	46	95	22.61

20 Example 5

5.0 g of the PPIC described above in Example 4 was dissolved in 20 g of acetone (concentration of 20% PPIC by weight). This solution was then nebulized at a flow rate of 4.0 ml/min over 500 ml of bath of ethanol at -10°C as described in Example 4. After preparation of the PPIC particles in the bath, 500 ml of deionized water was added to the bath, and the bath was then brought up to 0°C. The bath was then stirred for 30 min, brought up to 20°C, and stirred for an additional 30 min. The PPIC particles were then recovered by filtration and dried under vacuum at room temperature. An analysis of the resulting particles is listed in Table II.

TABLE II

Example	D 0.1 (μm)	D 0.5 (μm)	D 0.9 (μm)	Specific Area (m <sup>2</sup> /g)
#4	13	46	95	22.61
#5	50	99	180	0.11

As shown in Table II, different morphologies of particles were obtained. The particles of Example 4 were larger and had a lower specific area. As indicated by electron scanning microscope, the particles obtained in Example 4 were also more porous, likely because of the frozen water which remained in the particles upon precipitation. When the bath dispersion was returned to room temperature, the water thawed and flowed into the ethanol bath, leaving open channels in the microparticles. Consequently, these particles were more brittle and generated fragments of small size.

Example 6

A solution of PPIC described above in Example 5 was nebulized at 2.5 ml/min over 1.5 liters of deionized water at 0°C. An analysis of the resulting particle sizes is listed in Table III.

Example 7

A solution of PPIC described above in Example 5 was nebulized at 2.5 ml/min over 1.5 liters of ethanol at -10°C. An analysis of the resulting particle sizes is listed in Table III.

TABLE III

Example	D 0.1 (μm)	D 0.5 (μm)	D 0.9 (μm)	Specific Area (m <sup>2</sup> /g)
6	53.4	154.3	329.1	n/a
7	42.4	87.2	170.1	0.20

Example 8

Two solutions of PPIC are prepared in acetone as described above in Example 5. The first solution has a PPIC concentration of 15% while the second solution has a PPIC concentration of 20%. The solutions are nebulized over a bath of ethanol at -10°C at flow rates of 2.5, 3.5, and 5.0 ml/min as described in Example 5. An analysis of the resulting particle size is listed in Table IV.

TABLE IV

Concentration	Feeding Rate (ml/min)	D 0.1 (μm)	D 0.5 (μm)	D 0.9 (μm)	Specific Area (m <sup>2</sup> /g)
15%	2.5	35.9	81.6	191.1	4.455
15%	3.5	34.4	80.2	188.3	8.336
15%	5.0	49.4	163.6	397.8	n/a
20%	2.5	33.3	73.8	145.6	0.199
20%	3.5	50.8	112.7	241.9	0.579
20%	5.0	108.3	219.1	395.9	n/a

Analysis of the particles using scanning electron microscope revealed that the particle size and the specific area increased with the increase in feeding rate.

Example 9

5.0 g of PPIC microparticles of Example 4 was dissolved in 45 g of acetone (concentration 10% by weight). The solution was then added dropwise over a vigorously stirred 500 ml n-hexane at room temperature. The n-hexane solution turned cloudy as particles of PPIC precipitated. The PPIC was removed by filtration and dried under vacuum at room temperature.

Example 10

In a jacketed reactor, 3.0 g of the PPIC microparticles described in Example 2 was dispersed in a vigorously stirred 250 ml of 12,500 cs medical grade silicon oil (Dow Corning, Midland, MI) (of 1% PPIC by weight). After the stirring, the mixture was then heated to 120°C, which is above the Tg of 55°C for the PPIC, and kept at this temperature for 30 minutes. During this heating, the isolated individual particles melted to form spherical droplets. The dispersion was then cooled to 20°C and then diluted with 1250 ml of hexane. The microspheres subsequently hardened, were recovered by filtration, were rinsed with fresh hexane, and were finally dried under vacuum. The characteristics of the obtained microspheres are reported in Table V. The final microspheres had a small diameter as compared to those of Example 2 as a result of the compaction of the particles during melting.

TABLE V

25	Example	D 0.1 (μm)	D 0.5 (μm)	D 0.9 (μm)	Specific Area (m <sup>2</sup> /g)
#2		10	30	62	18.64
#7		2	10	47	<0.33

Example 11

0.2 g of the PPIC microparticles described in Example 2 were dispersed in 5 ml of deionized water and vigorously stirred with a vortex shaker. 100 ml microliters of dichloromethane (DCM) was then added over the stirred dispersion. The addition of a small amount of DCM caused a swelling of the surface of the PPIC particles. Stirring was kept at room temperature for 4 hours, allowing for evaporation of DCM and consequential hardening of the swollen surface of the particles. A scanning electron microscope showed that the resulting particles were of spherical shape with a smoother surface as compared to the starting material. Both the particle size distribution was narrowed, and the maximum particle size was reduced as a result of the increase in the density of the particles.

Example 12

One liter of sesame seed oil (Vitamins, Inc., Chicago, IL) was placed in a 2 liter, three necked flask immersed in a water bath. The oil was stirred at 600 rpm using a teflon stirring paddle connected to an overhead stirring motor. 500 mg of the surfactant, soybean lecithin, (Sigma Chemicals, St. Louis, MO) was added to the sesame seed oil, and the mixture was stirred for 10 min. 10 g of a PPIC formulation was then dissolved in 100 ml acetonitrile to give a clear solution. The PPIC compositions were made using Lanreotide<sup>TM</sup> conjugated with one of the following three polymers: 64/34/2 poly-DL-lactic-co-glycolic-D,L-malic acid copolymer (M.W. avg 6,000) (Composition 1); 74/24/2 poly-DL-lactic-co-glycolic-D,L-malic acid copolymer (M.W. avg 6,000) (Composition 2); and 98/2 poly-DL-lactic-co-D,L-malic acid copolymer (Composition 3).

This clear PPIC solution was added dropwise through a dropping funnel. When the addition was completed, the temperature of the external water bath was raised to 40°C, and the oil was left stirring for 20 h. One liter of hexane was then added to dilute the sesame seed oil,

and the oil was filtered through a medium fritted funnel. The microspheres collected in the filter funnel were further washed several times with 500 ml in total volume of hexane. The particles are dried at 36°C for two days under vacuum. Characteristics of the resulting

5           microspheres are presented in Table VI.

TABLE VI

	Composition	D 0.1 (μm)	D 0.5 (μm)	D 0.9 (μm)	Specific Area (m <sup>2</sup> /g)
10	1	13	28	57	0.1426
	2	13	25	59	0.1395
15	3	14	25	51	0.1480

Example 13

Reactor was loaded with monomers glycolide (Purac Biochem, Netherlands, 84.83 g), lactide (Purac Biochem, Netherlands, 210.67 g) and L(+)-tartaric acid (Riedel-de Haen, Seelze, Germany, article number 33,801, 4.50 g) and stannous 2-ethyl hexanoate (Sigma, St. Louis, Missouri, USA, article number S-3252) in toluene (Riedel-de Haen, Seelze, Germany) solution (0.1025 M, 4.34 ml). The L(+)-tartaric acid was previously dried over phosphorous pentoxide (Riedel-de Haen, Seelze, Germany) in an Abderhalden drying apparatus for 10 hours. The reactor (connected to pump *via* a liquid nitrogen trap) was then put under vacuum (0.04 mbar) with stirring for 50 minutes to remove toluene. The reactor, under an atmosphere of oxygen-free nitrogen (BOC gases, Dublin, Ireland, moisture content of 8 VPM), was then immersed in an oil bath (Temperature = 200°C) and stirring was increased to 125 rpm. Prior to immersion, a heating tape (Thermolyne type 45500, input control setting = 4) was placed on the

reactor lid. The time taken to completely melt the reactor contents was noted, typically 10 minutes for a load of 300 g at 200°C. Samples were taken every hour during synthesis and analyzed by GPC to determine the percentage residual monomer and to obtain values for average  
5 molecular weight by number (Mn) and by weight (Mw). Typical reaction times are of the order of 6 hours.

An amorphous copolymer was obtained comprising 66.21% lactide units, 33.11% glycolide units, and 0.68% tartaric acid units  
10 (66/33/1 PLGTA). The acid number of the titration was determined to 0.303 milliequivalents/g (meq/g; the normality of NaOH multiplied by the volume of NaOH solution required to neutralize one gram of polyester). The average number average molecular weight of the copolymer had a value of 10,250, the average weight molecular weight of the copolymer was 11,910 giving a Mw/Mn value of 1.16.

15 41.32 g of the above 10,000 g/mol 66/32/2 poly-L-lactic-co-glycolic-co-L(+)tartaric acid copolymer (acid number = 0.303 meq/g) was dissolved in 165.52 g of acetone (Riedel-de Haen, Seelze, Germany) by sonication in a Branson sonication bath (Branson, Danbury, Connecticut, USA) to give a solution with a concentration of  
20 19.98% PLGTA by weight.

To this solution was added 37.6 ml 0.2 N sodium carbonate  
25 (Aldrich, Gillingham, Dorset, UK) thus providing a 1.2 times excess of sodium over copolymer carboxyl groups. The solution was left to stir for 30 minutes to aid sodium salt formation. It was then fed to an atomizer nozzle at 8.0 ml/min using a Masterflex pump (Cole Parmer, Barrington, Illinois, USA). The solution was nebulized into a 6 L jacketed reactor containing 2 L of de-ionized water cooled to 2.5°C using a circulation bath (Huber, Offenburg, Germany). This water was stirred to 350 rpm using a 4-blade paddle linked to a stirrer motor.

30 Once nebulization was complete, the dispersion was placed in 6 centrifuge bottles and spun at 5000 rpm for 30 minutes in a Sorvall centrifuge (DuPont Sorvall Products, Wilmington, Delaware, USA).

The resultant centrifuge cakes were resuspended in de-ionized water and re-spun. The supernatant was discarded and the cakes were frozen in a freezer overnight before being dried in a small-scale lyophilizer (Edwards, Crawley, West Sussex, UK) the next day. 33.16 g of washed 5 copolymer were recovered representing a yield of 80.24%.

4.92 g of the above 10,000 g/mol 66/33/1 poly-L-lactic-co-glycolic-co-D,L-tartaric acid copolymer (66 percent L-lactic acid, 33 percent glycolic acid, and 1 percent tartaric acid) was dissolved in 11.58 g of acetonitrile (Ridel-de Haen, Seelze, Germany; HPLC grade) 10 by sonication in a Branson sonication bath (Branson, Danbury, CT, USA) and stirred on a stir-plate producing a solution with a concentration of 29.82% of PLGTA by weight.

This copolymer/acetonitrile solution was fed from a glass reservoir through an atomizer nozzle using an FMI revolving piston pump (FMI, Oyster Bay, NY, USA) set at 2.0 ml/min. The atomizer output power was set at 50 W with an amplitude of 80%. The solution was nebulized into a 6L jacketed reactor containing 1.5 L of general purpose reagent isopropyl alcohol (Labscan, Dublin, Ireland), cooled to -70°C by solid CO<sub>2</sub> pellets (AIG, Dublin, Ireland), and stirred at 300 rpm with a 4-blade paddle linked to a stirrer motor. The temperature 15 of the isopropyl alcohol remained at or close to -70°C throughout nebulization which lasted for approximately 8 minutes.

Once nebulization was complete, the dispersion was allowed to warm to 10°C of its own accord over a period of 5.5 hours. It was 20 then filtered over a Whatman No. 1 filter paper (9 cm diameter) with the aid of a vacuum. The filter paper and cake were placed in a desiccator along with silica gel drying chips and a vacuum was pulled through an automatic refrigeration trap at -110°C. After 24 hours 4.24 g of material were recovered. An analysis of the resulting particles is 25 listed in Table VII.

TABLE VII

	Example #	D 0.1 ( $\mu\text{m}$ )	D 0.5 ( $\mu\text{m}$ )	D 0.9 ( $\mu\text{m}$ )	Specific Area ( $\mu^2/\text{g}$ )
5					
	13	31	68	139	0.16

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the claims.

Claims:-

1. A method of making microparticles of a sustained release ionic conjugate containing a free carboxyl group-containing biodegradable polymer and a free amino group-containing drug which are ionically bonded to each other, the method comprising:

5 obtaining a first solution in which said conjugate is dissolved, wherein said solution comprises acetone, acetonitrile, ethyl acetate, tetrahydrofuran, or glyme;

10 mixing said first solution with a first liquid to form a first dispersion, wherein said first liquid is miscible with said first solution, and said conjugate is not soluble in said first liquid and precipitates out of said first dispersion; and

isолating said conjugate from said first dispersion.

15 2. A method according to claim 1, wherein said first solution is added to said first liquid as small droplets.

3. A method according to claim 2, wherein said first solution is added to said first liquid through an atomizing nozzle.

4. A method according to any one of claims 1-3, wherein said drug is a peptide.

20 5. A method according to any preceding claim, wherein said biodegradable polymer is a polyester made of lactic acid, e-caprolic acid, glycolic acid, trimethylene carbonate, or p-dioxanone; or a copolymer thereof.

25 6. A method according to any preceding claim, wherein said drug is soluble in said first liquid.

7. A method according to any preceding claim, wherein said biodegradable polymer is a polyester comprising lactic acid, or glycolic acid; or a copolymer thereof.

5 8. A method according to claim 7, wherein said polyester further contains malic acid, tartaric acid, citric acid, succinic acid, or glutaric acid.

9. A method according to any one of claims 4-8, wherein said peptide is a somatostatin or LHRH.

10 10. A method according to any preceding claim, wherein said first liquid is alcohol or water; or a mixture thereof.

11. A method according to claim 10, wherein said first liquid is ethanol maintained between about 0°C and -30°C or isopropyl alcohol maintained between about 0°C and -70°C.

15 12. A method according to any preceding claim, wherein said first solution contains acetone or acetonitrile.

13. A method according to any preceding claim, wherein said first solution is obtained by:

dissolving said biodegradable polymer in a second liquid to form a second solution;

20 dissolving said drug in a third liquid to form a third solution, wherein said third liquid is miscible with said first liquid and said second liquid; and

25 mixing said second solution and said third solution to form said first solution, wherein said mixing causes said drug to ionically bond to said biodegradable polymer and form said conjugate in said first solution.

14. A method according to claim 13, wherein NaOH or KOH is added to the second solution prior to mixing said second solution and said third solution.

5 15. A method according to claim 13 or 14, wherein said second liquid is acetone; and said third liquid is water or acetone; or a mixture thereof.

10 16. A method according to any of claims 1-12, wherein said first solution is obtained by dissolving said biodegradable polymer and said drug in a second liquid to form said first solution, thereby forming said conjugate in said first solution.

17. A method according to claim 16, wherein said second liquid is acetone or a mixture of acetone and water.

15 18. A method according to claim 17, wherein said biodegradable polymer is first dissolved in said second liquid, a base is then added to said second solution, and said drug is subsequently dissolved in said second liquid.

19. A method according to any preceding claim, wherein said conjugate is isolated by centrifuging or filtering said first dispersion.

20 20. A method according to claim 19, wherein said first solution is partially or completely evaporated from said first dispersion prior to isolation of said conjugate.

21. A method according to claim 20, wherein said isolated conjugate is mixed with an aqueous mannitol solution prior to vacuum drying.

25 22. A method of spherifying a sustained release ionic conjugate comprising a biodegradable polymer containing a free carboxyl group-containing biodegradable polymer and a free amino group-containing drug which are ionically bonded to each other, said method comprising:

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mixing said conjugate with a first liquid to form a first dispersion, wherein said conjugate has the shape of a microparticle and is not soluble in said first liquid;

5 heating said first dispersion to a temperature greater than the Tg or Tm of said conjugate;

cooling said first dispersion below the Tg or Tm of said conjugate;

10 mixing said first dispersion with a second liquid to form a second dispersion, wherein said second liquid is miscible with said first liquid and said conjugate is not soluble in said second liquid; and

isolating said conjugate from said second dispersion.

23. A method according to claim 22, wherein said conjugate has the shape of a microcapsule which has an average diameter of between 5 µm to 200 µm prior to mixing with said first liquid and said first dispersion is stirred prior to said heating or cooling.

15 24. A method according to claim 22 or 23, wherein said biodegradable polymer is a polyester made of lactic acid or glycolic acid; or a copolymer thereof.

20 25. A method according to any one of claims 22-24, wherein said drug is a peptide.

26. A method according to any one of claims 22-25, wherein said first liquid is an oil and said second liquid is hexane.

27. A method according to any one of claims 22-26, further comprising:

25 rinsing said isolated conjugate with said second liquid; and

vacuum drying said rinsed conjugate.

28. A method according to claim 27, wherein said isolated conjugate is mixed with an aqueous mannitol solution prior to vacuum drying.

5        29. A method of spherifying a sustained release ionic conjugate containing a free carboxyl group-containing biodegradable polymer and a free amino group-containing drug which are ionically bonded to each other, said method comprising:

dissolving said conjugate in a first liquid to form a first solution;

10      stirring said first solution with a second liquid to form a first dispersion, wherein said second liquid is immiscible with said first solution;

evaporating said first liquid from said first dispersion to precipitate said conjugate from said first dispersion; and

15      isolating said precipitate conjugate from said first dispersion.

30. A method according to claim 29, wherein said first solution is added to said second liquid as small droplets.

31. A method according to claim 29 or 30, wherein said first liquid is acetonitrile and said second liquid is an oil.

20      32. A method according to claim 31, wherein said oil is silicon oil, mineral oil, sesame oil, or a vegetable oil.

33. A method according to any one of claims 29-32, wherein said biodegradable polymer is a polyester comprising lactic acid or glycolic acid; or a copolymer thereof.

34. A method according to any one of claims 29-33, wherein said drug is a peptide.

35. A method according to any one of claims 29-34, further comprising rinsing said isolated conjugate with a third liquid which is miscible with said second liquid and not a solvent for said isolated conjugate.

36. A method according to claim 35, wherein said third liquid is hexane, heptane, or octane.

37. A method according to any one of claims 29-36, wherein said isolated conjugate is mixed with an aqueous mannitol solution prior to vacuum drying.

38. A method of making microparticles of a sustained release ionic conjugate, substantially as hereinbefore described and exemplified.

39. A method of spherifying a sustained release ionic conjugate, substantially as hereinbefore described and exemplified.

- 23 -

1        40. A method according to claim 8, wherein said  
2 polyester comprises lactic acid, glycolic acid and tartaric  
3 acid.

1        41. Biodegradable polymer comprising lactic acid,  $\epsilon$ -  
2 caproic acid, glycolic acid, trimethylene carbonate, p-  
3 dioxanone or a copolymer thereof and tartaric acid.

1        42. Microparticles comprising a biodegradable polymer  
2 according to claim 41.

1        43. Microparticles of a sustained release ionic  
2 conjugate comprising the biodegradable polymer according to  
3 claim 41 and a drug containing one or more free amino groups,  
4 wherein the polymer and drug are ionically bonded.

1        44. Microparticles according to claim 43 wherein said  
2 drug is selected from the group consisting of growth hormone  
3 releasing peptide luteinizing hormone-releasing hormone,  
4 adrenomedullin, growth hormone, somatostatin, bombesin,  
5 gastrin releasing peptide, calcitonin, bradykinin, galanin,  
6 melanocyte stimulating hormone, growth hormone releasing  
7 factor, amylin, tachykinins, secretin, parathyroid hormone,  
8 enkephalin, endothelin, calcitonin gene releasing peptide,  
9 neuromedins, parathyroid hormone related protein, glucagon,  
10 neuropeptides, adrenocorticotrophic hormone, peptide YY,  
11 glucagon releasing peptide, vasoactive intestinal peptide,  
12 pituitary adenylated cyclase activating peptide, motilin,  
13 substance P, neuropeptide Y and TSH or an analogue or a  
14 fragment thereof.

1        45. Microparticles according to claim 44 wherein said  
2 drug is somatostatin or LHRH or an analogue or a fragment  
3 thereof.

1       46. Microparticles according to claim 45 wherein said  
2 somatostatin analogue is D- $\beta$ -Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-  
3 Nh<sub>2</sub>.

1       47. The biodegradable polymer according to claim 41  
2 comprising lactic acid glycolic acid and tartaric acid.

1       48. Microparticles comprising a biodegradable polymer  
2 according to claim 47.

1       49. Microparticles of a sustained release ionic  
2 conjugate comprising the biodegradable polymer according to  
3 claim 47 and a drug containing one or more free amino groups,  
4 wherein the polymer and drug are ionically bonded.

1       50. Microparticles according to claim 49 wherein said  
2 drug is selected from the group consisting of growth hormone  
3 releasing peptide, luteinizing hormone-releasing hormone,  
4 adrenomedullin, growth hormone, somatostatin, bombesin,  
5 gastrin releasing peptide, calcitonin, bradykinin, galanin,  
6 melanocyte stimulating hormone, growth hormone releasing  
7 factor, amylin, tachykinins, secretin, parathyroid hormone,  
8 enkephalin, endothelin, calcitonin gene releasing peptide,  
9 neuromedins, parathyroid hormone related protein, glucagon,  
10 neurotensin, adrenocorticotrophic hormone, peptide YY,  
11 glucagon releasing peptide, vasoactive intestinal peptide,  
12 pituitary adenylated cyclase activating peptide, motilin,  
13 substance P, neuropeptide Y and TSH or an analogue or a  
14 fragment thereof.

1       51. Microparticles according to claim 50 wherein said  
2 drug is somatostatin or LHRH or an analogue or a fragment  
3 thereof.

1       52. Microparticles according to claim 51 wherein said  
2 somatostatin analogue is D- $\beta$ -Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-  
3 Nh<sub>2</sub>.

1       53. The biodegradable polymer according to claim 47  
2 wherein the ratio of lactic acid to glycolic acid to taratic  
3 acid is about 66 to about 33 to about 1, respectively.

1       54. Microparticles comprising a biodegradable polymer  
2 according to claim 53.

1       55. Microparticles of a sustained release ionic  
2 conjugate comprising the biodegradable polymer according to  
3 claim 53 and a drug containing one or more free amino groups,  
4 wherein the polymer and drug are ionically bonded.

1       56. Microparticles according to claim 55 wherein said  
2 drug is selected from the group consisting of growth hormone  
3 releasing peptide, luteinizing hormone-releasing hormone,  
4 adrenomedullin, growth hormone, somatostatin, bombesin,  
5 gastrin releasing peptide, calcitonin, bradykinin, galanin,  
6 melanocyte stimulating hormone, growth hormone releasing  
7 factor, amylin, tachykinins, secretin, parathyroid hormone,  
8 enkephalin, endothelin, calcitonin gene releasing peptide,  
9 neuromedins, parathyroid hormone related protein, glucagon,  
10 neuropeptidin, adrenocorticotrophic hormone, peptide YY,  
11 glucagon releasing peptide, vasoactive intestinal peptide,  
12 pituitary adenylylated cyclase activating peptide, motilin,  
13 substance P, neuropeptide Y and TSH or an analogue or a  
14 fragment thereof.

1       57. Microparticles according to claim 56 wherein said  
2 drug is somatostatin or LHRH or an analogue or a fragment  
3 thereof.

1       58. Microparticles according to claim 57 wherein said  
2 somatostatin analogue is D- $\beta$ -Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-  
3 NH<sub>2</sub>.

1       59. The biodegradable polymer according to claim 47  
2 wherein the ratio of lactic acid to glycolic acid to taratic  
3 acid is about 66 to about 32 to about 2, respectively.

1       60. Microparticles comprising a biodegradable polymer  
2 according to claim 59.

1       61. Microparticles of a sustained release ionic  
2 conjugate comprising the biodegradable polymer according to  
3 claim 60 and a drug containing one or more free amino groups,  
4 wherein the polymer and drug are ionically bonded.

1       62. Microparticles according to claim 61 wherein said  
2 drug is selected from the group consisting of growth hormone  
3 releasing peptide, luteinizing hormone-releasing hormone,  
4 adrenomedullin, growth hormone, somatostatin, bombesin,  
5 gastrin releasing peptide, calcitonin, bradykinin, galanin,  
6 melanocyte stimulating hormone, growth hormone releasing  
7 factor, amylin, tachykinins, secretin, parathyroid hormone,  
8 enkephalin, endothelin, calcitonin gene releasing peptide,  
9 neuromedins, parathyroid hormone related protein, glucagon,  
10 neuropeptid Y, adrenocorticotrophic hormone, peptide YY,  
11 glucagon releasing peptide, vasoactive intestinal peptide,  
12 pituitary adenylated cyclase activating peptide, motilin,  
13 substance P, neuropeptide Y and TSH or an analogue or a  
14 fragment thereof.

1       63. Microparticles according to claim 62 wherein said  
2 drug is somatostatin or LHRH or an analogue or a fragment  
3 thereof.

1       64. Microparticles according to claim 63 wherein said  
2 somatostatin analogue is D- $\beta$ -Nal-c[Cys-Tyr-D-Trp-Val-Cys]-Thr-  
3 NH<sub>2</sub>.

1       65. A method according to claim 21 wherein said  
2 polyester comprises lactic acid, glycolic acid and tartaric  
3 acid.

1        66. A method according to claim 28 wherein said  
2 polyester comprises lactic acid, glycolic acid and tartaric  
3 acid.

1        67. A method according to claim 37 wherein said  
2 polyester comprises lactic acid, glycolic acid and tartaric  
3 acid.

## **SUSTAINED RELEASE IONIC CONJUGATE**

## **ABSTRACT**

**A method of spherifying a sustained release ionic conjugate which contains a free carboxyl group-containing biodegradable polymer and a free amino group-containing drug which are ionically bonded to each other.**

Docket No.  
BPC034

## Declaration and Power of Attorney For Patent Application

### English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

#### SUSTAINED RELEASE IONIC CONJUGATE

the specification of which

(check one)

is attached hereto.

was filed on April 22, 1997 as United States Application No. or PCT International Application Number PCT/IE97/00030

and was amended on \_\_\_\_\_  
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

#### Prior Foreign Application(s)

Priority Not Claimed

960308 (Number)	Ireland (Country)	23 April 1996 (Day/Month/Year Filed)	<input type="checkbox"/>
 (Number)	 (Country)	 (Day/Month/Year Filed)	<input type="checkbox"/>
 (Number)	 (Country)	 (Day/Month/Year Filed)	<input type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

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(Application Serial No.)

(Filing Date)

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(Application Serial No.)

(Filing Date)

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(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

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PCT/IE97/00030

22 April 1997

Pending

(Application Serial No.)

(Filing Date)

(Status)

(patented, pending, abandoned)

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(Application Serial No.)

(Filing Date)

(Status)

(patented, pending, abandoned)

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(Application Serial No.)

(Filing Date)

(Status)

(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name and registration number)

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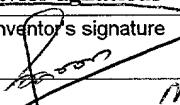
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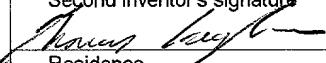
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